Advanced Drug Delivery Reviews 79–80 (2014) 3–18

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Matrigel: From discovery and ECM mimicry to assays and models for cancer research ☆

Gabriel Benton [a], Irina Arnaoutova [a], Jay George [a], Hynda K. Kleinman [b,*], Jennifer Koblinski [c]

[a] Trevigen Inc., Gaithersburg, MD 20877, USA
[b] The George Washington University School of Medicine, Washington, DC 20037, USA
[c] Virginia Commonwealth University, Richmond, VA 23220, USA

ARTICLE INFO

Available online 2 July 2014

Keywords:
3D culture
Spheroids
Invasion assay
Xenograft
Basement membrane
Extracellular matrix
Angiogenesis
Dormancy assay

ABSTRACT

The basement membrane is an important extracellular matrix that is found in all epithelial and endothelial tissues. It maintains tissue integrity, serves as a barrier to cells and to molecules, separates different tissue types, transduces mechanical signals, and has many biological functions that help to maintain tissue specificity. A well-defined soluble *b*asement *m*embrane *e*xtract, termed BME/Matrigel, prepared from an epithelial tumor is similar in content to authentic basement membrane, and forms a hydrogel at 24–37 °C. It is used in vitro as a substrate for 3D cell culture, in suspension for spheroid culture, and for various assays, such as angiogenesis, invasion, and dormancy. In vivo, BME/Matrigel is used for angiogenesis assays and to promote xenograft and patient-derived biopsy take and growth. Studies have shown that both the stiffness of the BME/Matrigel and its components (i.e. chemical signals) are responsible for its activity with so many different cell types. BME/Matrigel has widespread use in assays and in models that improve our understanding of tumor biology and help define therapeutic approaches.

Contents

Abbreviations: BME, basement membrane extract; EGF, epidermal growth factor; TGF beta, transforming growth factor beta; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial cell growth factor; NIH, National Institutes of Health; PLF, proliferin; EHS, Engelbreth–Holm–Swarm tumor; 3D, 3-dimensional; AKT, protein kinase B; DIVAA, directed in vivo angiogenesis assay.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Engineering of Tumor Microenvironments".

* Corresponding author at: Trevigen Inc., 8405 Helgerman Ct, Gaithersburg, MD 20877, USA.
E-mail address: hyndakk@aol.com (H.K. Kleinman).

http://dx.doi.org/10.1016/j.addr.2014.06.005
0169-409X/

1. Introduction

1.1. Basement membrane structure and function

This chapter will focus on the use of the *b*asement *m*embrane *e*xtract (BME/Matrigel) with tumor cells/tissues in cancer biology with an emphasis on recent findings and its uses for testing potential cancer therapeutics as well as testing for the activity of regenerative molecules [1–5]. BME/Matrigel has had a large impact on both angiogenesis and tumor biology research both in vitro and in vivo as a tool in widely used and critical functional assays and in modeling of the tumor microenvironment. These uses/models have great importance in drug screening.

Basement membranes are thin membrane-like, acellular extracellular matrices which underlie epithelial and endothelial cells, surround smooth muscle and fat cells, and comprise the matrix of most tumors which are generally of epithelial origin [3,6,7]. The major components of basement membranes are laminins, collagen IV, heparan sulfate proteoglycan, and various growth factors, cytokines, chemokines, and proteases. The amount and type of these components vary depending on the stage of development and on the tissue type or in the case of tumors on the tumor type and level of dedifferentiation. These components generally interact with each other via specific interactions to form an organized matrix that helps to separate different tissue types, support tissue integrity, act as a mechanosensor, maintain cell differentiation, serve as a barrier to proteins and cells, and filter waste in the kidney [3,4,8]. The basement membrane acts as a storage depot for growth factors and enzymes [9,10]. When basement membranes are degraded by tumor cells, these factors are released and further enhance the tumor cells' ability to grow and spread. The components laminin and collagen IV have many functions, including promoting cell adhesion and migration, and specific cell surface receptors have been identified which mediate these interactions. Laminin, in particular, has multiple adhesion sites and interacts with various cell surface receptors. These different interactions result in a variety of positive and negative effects on tumor biology [4].

1.2. BME/Matrigel timeline of discovery and uses

An extract of the EHS tumor has been found to contain the major basement membrane components and has many in vitro and in vivo uses [3]. The extract is mainly composed of laminin-111 and when in high concentration (greater than 4 mg/ml) it will gel at 24–37 °C. The tumor was originally isolated from a wild mouse (Table 1) and then transferred to a mouse tumor repository maintained in the USA. The tumor was tentatively identified as a chondrosarcoma based on the abundance of extracellular matrix observed at the histological level. However, when researchers at NIH began to study its components, it became clear that the matrix was comprised of basement membrane components. Having a plentiful source of basement membrane greatly facilitated the identification and characterization of the component proteins. Furthermore, the matrix could serve as a barrier to tumor cell invasion to study tumor cells' aggressiveness, since it is known that many types of tumor cells must degrade the tissue basement membrane in order to invade and metastasize. A quantitative, reliable, rapid, and simple in vitro assay that measures this invasive activity was developed [1]. A number of additional in vitro studies showed its potential role in stimulating cell adhesion, cell differentiation, tissue organization, and tissue explant growth. For example, breast epithelial cells ceased proliferating, formed acinar-like structures, and showed a large increase in the production of milk proteins when grown on BME/Matrigel. Similarly, when grown on BME/Matrigel, bone cells ceased proliferation and formed canaliculi [11–15]. Bone cell differentiation studies also showed the importance of specific growth factors when the growth factor reduced BME/Matrigel was supplemented with various growth factors. In contrast, tumor cells grown in 3D culture on BME/Matrigel continued to proliferate and showed invasive behavior consistent with their malignancy [16,17]. Interestingly, some dormant cells remained quiescent on this matrix; whereas, they proliferated well on tissue culture plastic [18,19]. Finally, a major finding was that BME/Matrigel has important uses in vivo for promoting tumor cell "take" and growth as well as patient-derived tumor biopsy growth in mouse models [20]. As few as one cell can give rise to a viable tumor when co-injected with BME/Matrigel into immune deficient animals [21]. BME/Matrigel is also widely used for tissue and cell transplants, including stem cells, and greater survival and tissue regeneration are observed [22–24].

1.3. Components of BME/Matrigel

The composition of BME/Matrigel is similar to a conserved, early developmental basement membrane that facilitates microtissue organization across different tissue types and different species. BME/Matrigel has been well-defined based on an in depth proteonomics analysis [25]. Analysis of the components in BME/Matrigel indicates that the matrix is comparable to embryonic basement membrane since the major components are laminin-111, collagen IV, entactin, and heparan sulfate proteoglycan which provide both structural and signal transduction functions [3]. In addition to the major components, several growth factors have been identified, including FGF, EGF, TGF beta, IGF, and PDGF, and selective deletion of these growth factors from BME/Matrigel has shown their functional significance with various cells types [26,27]. Furthermore, proteases have also been identified in BME/Matrigel [10]. BME/Matrigel is commercially available as a soluble extract with different compositions that contribute to its biological activity (Trevigen Inc., Gaithersburg, MD, Beckton Dickinson, Franklin Lakes, NJ). It is available with reduced growth factors which allows for defining the growth factor requirements for different cells. This is particularly important in the angiogenesis/tube assay when characterizing the activity of specific angiogenic factors. BME/Matrigel is now also available with increased

Table 1
Timeline of BME/Matrigel development and uses.

1944 Wild mouse caught by Dr. Engelbreth–Holm in Sweden
1961 Tumor transferred to Dr. Swarm USA (EHS tumor)
1972 Tumor transferred to George Martin, NIH: model for cartilage matrix
1973 EHS tumor redefined as basement membrane tumor
1974 Laminin-111 isolated from EHS tumor
1979 Heparan sulfate proteoglycan isolated from EHS tumor
1987 Invasion assay published, paper included 3D culture of tumor cells
1988 Notochord development: first use with stem cell explants in 3D culture
1989 Endothelial cell tube formation on BME
1990 Successful use for tumor xenograft
1992 Subcutaneous angiogenesis plug assay developed
1993 First orthotopic use for tumor xenograft
1994 Coinjection: tumor cells and fibroblasts + BME increased tumor growth
2001 Feeder free-growth of human embryonic stem cells
2006 BME first added to spheroid culture and found to promote compact spheroids
2008 Dormancy demonstrated with tumor-derived dormant cells on BME/Matrigel matrix
2008 One tumor cell + BME/Matrigel gives rise to a tumor in mice
2009 Tumorgraft: personalized medicine with human biopsies + BME/Matrigel in mice